Leptin and Norepinephrine Plasma Concentrations During Glucose Loading in Normotensive and Hypertensive Obese Women

Francesco Corica, Andrea Corsonello, Riccardo Ientile, Tiziana De Gregorio, Alba Malara, Antonio Artemisia, and Michele Buemi

We performed this study to investigate whether changes in plasma glucose, insulin, and norepinephrine concentrations during an oral glucose tolerance test (OGTT) are associated with changes in plasma leptin levels in normotensive and hypertensive obese women. Plasma insulin, glucose, norepinephrine, and leptin concentrations were evaluated at the baseline and during OGTT in normotensive women (NT-Ob, N = 24, mean age 38.3 ± 1.8 years, body mass index [BMI] 37.9 ± 1.1 kg/m²) and hypertensive (HT-Ob, N = 25, mean age 37.7 ± 1.9 years, BMI 39.4 ± 1.3 kg/m²) obese women, and in a group of normal-weight women (controls, N = 20, mean age 38.3 ± 1.3 years, BMI 23.1 ± 0.4 kg/m²). The OGTT caused a significant increase in plasma leptin concentrations in both NT-Ob and HT-Ob groups, whereas no such change was detectable in control subjects. Area under curve (AUC) for plasma leptin showed a direct correlation with norepinephrine AUC in both NT-Ob (r = 0.73, P = .001) and HT-Ob (r = 0.74, P = .001) group, which was still detectable in multivariate analysis (P = .014 and P = .017, respectively). Our study confirms that glucose loading increases circulating leptin concentrations in obese women, and demonstrates the existence of an association between leptin and norepinephrine changes during OGTT in both normotensive and hypertensive obese women. We hypothesize that this association may reflect the lack of leptin suppression by catecholamines or a direct leptin-induced sympathoactivation. These findings suggest that leptin could be relevant in the regulation of blood pressure in obese women. Am J Hypertens 2001;14: 619–626 © 2001 American Journal of Hypertension, Ltd.

Key Words: Leptin, norepinephrine, obesity, hypertension.
Methods

Subjects

The study was conducted on three groups of subjects, who had given their informed consent: 24 normotensive obese women (NT-Ob), 25 hypertensive obese women (HT-Ob), and 20 healthy women (control subjects), who were strictly matched for age. All obese subjects were recruited from the obese population attending the Outpatient’s Service for Prevention and Treatment of Obesity at the University Hospital of Messina, Italy. The healthy women who served as control subjects were selected from the staff of the hospital and were menstruating regularly.

Exclusion criteria included pregnancy, severe hypertension, cardiovascular disease, left ventricular hypertrophy, renal disease, and renal failure (serum creatinine > 1.4 mg/dL), diabetes mellitus, electrolyte imbalance, smoking habits, and alcohol abuse or psychiatric problems. Subjects who were on treatment with drugs known to modify SNS activity were also excluded; in particular, those on β-blockers were excluded. Hypertensive status in the HT-Ob group had been recently diagnosed, and the hypertensive obese subjects were enrolled in the study before they started any antihypertensive therapy. Other endocrine and metabolic diseases were ruled out on the basis of physical examination and laboratory tests.

The subjects were defined as obese according to the criteria of Garrow and Webster (body mass index [BMI] > 30 kg/m²). Subjects were considered lean when BMI values were < 25 kg/m². Body height was measured without shoes to the nearest 0.5 cm; body weight was measured without clothes to the nearest 0.1 kg; BMI was measured as weight/(height squared) (kg/m²); waist circumference was measured midway between the lower rib margin and the iliac crest; and hip circumference was determined as the widest circumference measured over the great trochanters. The waist-to-hip ratio (WHR) was then calculated. Hypertension was defined as a supine reading > 140/90 mm Hg on at least three separate measurements at 1-week intervals according to the indications of JNC-VI.

Measurements

All laboratory tests were performed in the follicular phase. In all subjects, after an overnight fasting, an OGTT was performed. Venous blood samples were obtained in the supine position 30 min before and every 30 min after ingestion of 75 g/200 mL oral glucose solution for 3 h, from the cubital vein of the arm using a cannula that had been placed at −30 min, and maintained by slow infusion of saline solution (10 drops/min). In all subjects studied OGTT was started between 8:00 and 8:30 AM. Blood samples for the assay of plasma glucose (AutoAnalyzer, Beckman, Milan, Italy), insulin (IRI, RIA kit, Insulin Solid Phase, Diagnostic Products, Los Angeles, CA), norepinephrine (HPLC, Diaman DM Bio-Rad, Segrate, Milan, Italy), and leptin (Human Leptin RIA kit, Linco Research Inc., St. Charles, MO) were collected under EDTA (10% acid citrate, dextrose ACD) and were centrifuged at 4°C within 1 h. Plasma was then transferred into capped poly-styrene round-bottom tubes and frozen at −80°C until the assay.

Arterial blood pressure was measured with a Riva-Rocci sphygmomanometer (Zenith, Rome, Italy) with an appropriately large cuff in obese subjects. The first measurement was excluded and the average of the following three measurements, taken at 3-min intervals, was considered. Mean blood pressure was calculated by the sum of diastolic blood pressure plus one third of pulse pressure.

Statistical Analysis

All statistical procedures were performed using SPSS for Windows release 7.5 (SPSS, Chicago, IL) statistical software package. The statistical analysis was made using the one-way analysis of variance with the Scheffe´ post hoc test for multiple comparisons for the evaluation of the difference between groups, and the t test for paired data to evaluate the differences of the mean at each time point from the baseline. Areas under the curve (AUC) for all parameters considered were calculated by the trapezoidal rule. The relationships among AUC for variables studied were evaluated using the Pearson correlation coefficient and multiple linear regression analysis. Data were expressed as means ± SE. Two-tailed values of P < .05 were considered statistically significant.

Results

General characteristics of the subjects studied are reported in Table 1. Baseline plasma glucose was significantly higher in hypertensive obese women with respect to controls, whereas plasma insulin, norepinephrine, and leptin at time 0′ were higher in the NT-Ob and HT-Ob groups when compared to those in lean subjects. Furthermore, baseline norepinephrine and leptin plasma concentrations were significantly higher in the HT-Ob than in the NT-Ob group (Table 1). Baseline plasma leptin concentrations were directly related to BMI in all the groups studied, whereas a negative correlation between fasting leptin levels and WHR was detectable in both the lean and obese groups. In additional, fasting leptin levels were positively correlated with systolic and mean blood pressure in the HT-Ob group. Furthermore, baseline leptin was positively correlated with fasting insulin and norepinephrine plasma concentrations in both NT-Ob and HT-Ob groups, whereas a negative correlation between fasting leptin and norepinephrine was observed in normal-weight controls (Table 2).

The oral glucose loading caused a significant increase in glucose, insulin, and norepinephrine plasma concentrations in all of the groups studied. However, the OGTT-induced increase in circulating glucose, insulin, and norepinephrine was higher in obese women than in the healthy control subjects. According to the plasma glucose
Table 1. General characteristics of the subjects studied

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (N = 20)</th>
<th>NT-Ob (N = 24)</th>
<th>HT-Ob (N = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>38.3 ± 1.2</td>
<td>38.3 ± 1.8</td>
<td>37.7 ± 1.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1 ± 0.4</td>
<td>37.9 ± 1.1*</td>
<td>39.4 ± 1.3*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.79 ± 0.01</td>
<td>0.90 ± 0.02*</td>
<td>0.91 ± 0.01*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.74 ± 0.10</td>
<td>5.08 ± 0.12</td>
<td>5.32 ± 0.18†</td>
</tr>
<tr>
<td>HDL cholesterol (%)</td>
<td>52.0 ± 1.1</td>
<td>40.2 ± 1.5*</td>
<td>39.6 ± 1.4*</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.73 ± 0.06</td>
<td>1.08 ± 0.06†</td>
<td>1.15 ± 0.07*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>121.5 ± 1.3</td>
<td>125.8 ± 1.6</td>
<td>161.2 ± 3.0*§</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>73.2 ± 1.5</td>
<td>76.7 ± 1.3</td>
<td>91.8 ± 1.6*§</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>89.3 ± 1.1</td>
<td>93.0 ± 1.1</td>
<td>114.9 ± 1.8§</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>4.25 ± 0.05</td>
<td>4.55 ± 0.15</td>
<td>4.97 ± 0.13*</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/L)</td>
<td>50.1 ± 3.9</td>
<td>107.6 ± 5.7*</td>
<td>117.1 ± 8.4*</td>
</tr>
<tr>
<td>Fasting plasma norepinephrine (pmol/L)</td>
<td>54.4 ± 3.5</td>
<td>121.1 ± 5.3*</td>
<td>139.2 ± 5.8 §§</td>
</tr>
<tr>
<td>Fasting plasma leptin (ng/mL)</td>
<td>15.3 ± 1.4</td>
<td>34.1 ± 3.0*</td>
<td>41.8 ± 2.1*</td>
</tr>
</tbody>
</table>

NT-Ob = normotensive obese patients; HT-Ob = hypertensive obese patients; BMI = body mass index; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure.

Data are mean ± SEM.

* P < .001,
† P < .01,
‡ P < .05 v controls;
§ P < .001,
|| P < .05 v NT-Ob.

time curves (Fig. 1), some obese women showed an impaired glucose tolerance. In fact, plasma glucose at 2 h was > 7.8 mmol/L in six of 24 subjects in the NT-Ob group (25%) and in seven of 25 subjects (28%) in the HT-Ob group.
The OGTT caused a significant increase in plasma leptin concentrations in both NT-Ob and HT-Ob groups, whereas no such change was detectable in the control subjects. In addition, the rise in plasma norepinephrine and leptin induced by glucose administration was significantly

Table 2. Simple correlations between fasting plasma leptin and anthropometric and metabolic variables in the groups studied

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (N = 20)</th>
<th>NT-Ob (N = 24)</th>
<th>HT-Ob (N = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>r = 0.144</td>
<td>r = 0.171</td>
<td>r = 0.333</td>
</tr>
</tbody>
</table>
P                                         | P = NS            | P = NS         | P = NS        |
| BMI (kg/m²)                             | r = 0.633         | r = 0.721      | r = 0.794     |
P                                         | r = 0.002         | r = 0.001      | r = 0.001     |
| WHR                                     | r = −0.596        | r = −0.448     | r = −0.495    |
P                                         | r = 0.006         | r = 0.036      | r = 0.012     |
| Total cholesterol (mg/dL)               | r = 0.187         | r = 0.272      | r = 0.192     |
P                                         | r = 0.198         | r = 0.038      | r = −0.158    |
| HDL cholesterol (%)                     | r = 0.142         | r = 0.129      | r = 0.029     |
P                                         | r = 0.006         | r = 0.001      | r = 0.012     |
| Triglycerides (mg/dL)                   | r = −0.221        | r = −0.218     | r = 0.469     |
P                                         | r = 0.343         | r = 0.354      | r = 0.417     |
| SBP (mm Hg)                             | r = 0.197         | r = 0.327      | r = 0.281     |
P                                         | r = 0.343         | r = 0.195      | r = 0.417     |
| DBP (mm Hg)                             | r = 0.197         | r = 0.327      | r = 0.281     |
P                                         | r = 0.343         | r = 0.195      | r = 0.417     |
| MBP (mm Hg)                             | r = 0.197         | r = 0.327      | r = 0.281     |
P                                         | r = 0.343         | r = 0.195      | r = 0.417     |
| Fasting plasma glucose (mmol/L)         | r = 0.343         | r = 0.593      | r = 0.426     |
P                                         | r = 0.343         | r = 0.593      | r = 0.426     |
| Fasting plasma insulin (pmol/L)         | r = −0.475        | r = 0.585      | r = 0.696     |
P                                         | r = 0.343         | r = 0.593      | r = 0.426     |

NS = not significant; other abbreviations as in Table 1.
higher in hypertensive when compared to normotensive obese women (Fig. 1). These results were also confirmed when the AUC for the above parameters were considered. In fact, both NT-Ob and HT-Ob groups showed a significant increase in glucose, insulin, norepinephrine, and leptin AUC when compared with those of control subjects, whereas norepinephrine AUC and leptin AUC were significantly higher in the HT-Ob than in the NT-Ob group (Fig. 2).

In bivariate correlation analysis, the leptin AUC showed a positive correlation with BMI and a negative correlation with WHR in all of the groups studied. In addition, leptin AUC was directly related to norepinephrine AUC in both the NT-Ob and HT-Ob groups. Multiple linear regression analysis, which was separately performed in each group considering leptin AUC as a dependent variable, showed that leptin AUC was directly associated with BMI in all groups studied. Furthermore, a positive association between leptin AUC and norepinephrine AUC was still detectable in both NT-Ob and HT-Ob groups after adjusting for age, BMI, WHR, glucose AUC, and insulin AUC (Table 3).

**Discussion**

Our study confirms that plasma leptin concentrations are higher in obese individuals than in lean subjects. Additionally, the negative correlation between circulating leptin and WHR seems to confirm the role of adipose tissue distribution in the regulation of leptin secretion in both lean and obese subjects.

Hypertensive obese women showed a significant increase in baseline circulating leptin when compared with that of normotensive obese women. Moreover, fasting leptin was directly correlated with systolic and mean blood pressure in the HT-Ob group. High plasma leptin levels have been found in hypertensive subjects, and circulating leptin has recently been reported to be associated with systolic blood pressure. Thus, our results further suggest that leptin may be involved in the pathophysiology of obesity-related hypertension.

Glucose loading led to a significant rise in plasma leptin levels in obese women, whereas no such finding was detectable in controls. Although some studies reported no changes in plasma leptin after the acute administration of carbohydrates, none of them investigated the changes in plasma leptin levels during OGTT. On the contrary, the results from the present study are in agreement with a recent report showing a significant rise in circulating leptin after glucose administration in obese women, but not in lean controls, and are consistent with the hypothesis that the increased leptin concentration after glucose loading may represent a regulatory mechanism enabling the resistance to leptin in the hypothalamus to be overcome. In this case, the lack of changes in circulating
leptin in lean women could be explained by the normal sensitivity to leptin in these subjects.

Among the possible factors involved in determining leptin changes during OGTT is the effect of insulin. Although, in the present study, fasting leptin is directly associated with fasting insulin in both normotensive and hypertensive obese women, leptin response to glucose loading was not correlated with insulin AUC. Several studies have shown a significant increase in plasma leptin concentrations after prolonged exogenous insulin infusion in rodents and humans, and patients with insulinoma have increased ob gene expression and circulating leptin levels. However, a euglycemic clamp with short term hyperinsulinemia (≤ 5 h) had no effect on circulating leptin levels, and supraphysiological hyperinsulinemia requires ≥ 4 h to produce a significant increase in circulating leptin levels. These data suggest that insulin could play a more relevant role in the long-term or diurnal regulation of leptin, and that the long-term stimulatory effect of insulin on leptin production is probably mediated by the trophic action of insulin on adipocytes. Our results are in agreement with the hypothesis that insulin is not able to acutely regulate leptin secretion during OGTT, and we speculate that other mechanisms could be involved in the pathophysiology of leptin response to glucose loading. Among these mechanisms consideration should be given to tumor necrosis factor-α (TNF-α), which could represent a common pathway in the pathophysiology of insulin-resistance and hyperleptinemia.

The lack of a significant correlation between leptin AUC and insulin AUC may also reflect gender-related differences in circulating leptin, which is known to be higher in women. The role of insulin in the regulation of leptin may be more relevant in men, as a direct correlation between insulin sensitivity and leptin has been reported in hypertensive men but not in women, and the suppressive action of testosterone may have a key role in determining this gender difference. Although only obese women (normotensive and hypertensive) and normal weight controls were enrolled in our study, Sheu et al studied a population of nondiabetic hypertensive patients and normotensive controls. They found a significant correlation between fasting plasma insulin and fasting leptin concentrations in both men and women, whereas steady-state plasma glucose, measured by insulin suppression test, was correlated with fasting plasma leptin only in men. Our results are in agreement with these findings, and also confirm the existence of a direct correlation between fasting plasma leptin and fasting insulin concentrations in obese women. However, we can not rule out that steady-state plasma glucose, a measurement of insulin sensitivity, may not be significantly correlated with fasting plasma leptin in obese women.

Leptin responsiveness to glucose loading could also involve other mediators such as gut peptides. In the last few years, ob mRNA and protein have been identified in rat gastric epithelium. More recently, leptin and its receptor have been found in the human stomach, and it has been demonstrated that intravenous infusion of pentagastrin or secretin is able to cause a significant rise in circulating leptin levels and leptin release into the gastric juice. Although much work is required to establish the physiological function of this source of leptin, one could speculate that leptin secreted from the stomach might...
contribute to the rise in circulating leptin observed during OGTT in our study.

Glucose administration caused a significant increase in plasma norepinephrine in all subjects studied, and obese subjects showed higher plasma norepinephrine levels than control subjects. Furthermore, in agreement with the findings reported by other investigators,\textsuperscript{30,31} plasma norepinephrine concentrations were higher in the HT-Ob than in the NT-Ob group. Hyperinsulinemia, which has been observed in obese patients, may have contributed to the rise in plasma norepinephrine concentrations by a direct insulin-mediated activation of the SNS.\textsuperscript{32} The finding of a direct association between leptin AUC and norepinephrine AUC in both normotensive and hypertensive obese women is particularly intriguing. Several reports suggest that a negative loop involving the SNS could contribute to the regulation of leptin synthesis,\textsuperscript{33,34} and our finding of a negative correlation between fasting leptin and norepinephrine in normal-weight control subjects is in agreement with this hypothesis. However, it has recently been reported that the hypersecretion of catecholamines is not associated with leptin suppression in patients with phaeochromocytoma, suggesting that the exposure of human adipose tissue to chronically elevated catecholamine concentration may induce the development of tolerance to catecholamines.\textsuperscript{35} Similarly, the chronic hyperactivity of the SNS is a well-known feature in obese individuals,\textsuperscript{36} and the concurrent rise in plasma norepinephrine and leptin that we observed in obese subjects may be partially due to the lack of leptin suppression by catecholamines.

Another pathophysiological explanation may be relevant to consider in the interpretation of our results. An increase in body weight or obesity is known to result in an activation of the SNS, which is also reflected in higher norepinephrine plasma concentrations in obese subjects.\textsuperscript{37} Leptin administration causes an increase in norepinephrine turnover and sympathetic nerve activity in rodents,\textsuperscript{6,7} which is mediated mainly by the ventromedial hypothalamus.\textsuperscript{38} Furthermore, central administration of leptin is able to cause a significant increase in circulating norepinephrine levels in primates.\textsuperscript{39} Finally, a direct correlation between plasma leptin concentrations and muscle sympathetic nerve activity has been found in human subjects.\textsuperscript{8} The sympathtoactivation induced by leptin may represent a compensatory mechanism to activate thermogenesis against the potentially deleterious effects of increases in adipose mass.\textsuperscript{9} However, the hyperactivity of the SNS is known to play a relevant role in the pathophysiology of the obesity-related hypertension.\textsuperscript{30,31} In light of these findings, we speculate that the association between leptin AUC and norepinephrine AUC observed in both normotensive and hypertensive obese women may reflect a direct action of leptin to increase circulating norepinephrine, and the stimulatory effect of leptin on the SNS may contribute to the pathophysiology of high blood pressure in obese subjects.

Interestingly, although plasma leptin levels in normotensive obese women were substantially higher than those

### Table 3.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>HT-Ob</th>
<th>NT-Ob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>0.14 NS</td>
<td>-0.03</td>
<td>0.21</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.64</td>
<td>0.958</td>
<td>0.81</td>
</tr>
<tr>
<td>WHR</td>
<td>0.60</td>
<td>-26.4</td>
<td>0.381</td>
</tr>
<tr>
<td>Glucose AUC (mmol/L/180 min)</td>
<td>0.35</td>
<td>-0.536</td>
<td>0.104</td>
</tr>
<tr>
<td>Insulin AUC (pmol/L/180 min)</td>
<td>-0.32</td>
<td>-0.047</td>
<td>0.73</td>
</tr>
<tr>
<td>Norepinephrine AUC (pmol/L/180 min)</td>
<td>0.108</td>
<td>0.057</td>
<td>0.076</td>
</tr>
</tbody>
</table>

AUC = area under curve (trapezoidal rule); other abbreviations as in Tables 1 and 2.
in control subjects, and although the leptin AUC correlated with the norepinephrine AUC in the NT-0b group, blood pressure in NT-Ob subjects was similar to that in the lean control subjects. Although leptin is able to increase sympathetic nerve activity, \textsuperscript{6,7,36,37} the \textit{ob} gene product has been shown to cause natriuresis and diuresis,\textsuperscript{40} and a recent study demonstrates that intravenous leptin administration produces a dose-dependent increase in nitrite/nitrate concentrations.\textsuperscript{41} Thus, the apparent discrepancy in blood pressure response to leptin and norepinephrine increase in the present study may be explained by the ability of leptin to influence different regulatory pathways with opposite effects on blood pressure control, with a pressor response mainly due to the SNS activation and a depressor response attributable to natriuretic/diuretic action and/or increased endothelial nitric oxide release.

Our study does have some limitations. The major limitation is the lack of a lean hypertensive control group. Furthermore, blood pressure and heart rate monitoring during OGTT was not available. Thus, the role of leptin in the pathophysiology of hypertension and the mechanisms linking leptin and the SNS activity need to be investigated further.

In conclusion, our study shows that glucose loading increases circulating leptin concentrations in obese women but not in lean control subjects. Furthermore, our results show the existence of an association between leptin and norepinephrine changes during OGTT in both normotensive and hypertensive obese women. This association may reflect the lack of leptin suppression by catecholamines and/or a direct leptin induced sympathoactivation. Further studies in this field are needed to better clarify the mechanisms involved in the pathophysiology of leptin response to glucose loading and the role of leptin in the development of hypertension in obese patients.

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References